A Study of Selenate Efflux from Human Placental Microvillus Membrane Vesicles

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Received August 24, 1987

KEY WORDS: selenate; human placenta; membrane vesicles.

ABBREVIATIONS: DIDS; 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonate.

Selenate efflux from human placental brush border membrane vesicles was studied using an ion-exchange column assay. Selenate efflux was found to be mediated almost exclusively by a temperature dependent DIDS-sensitive pathway. Chromate markedly inhibited selenate efflux: in contrast medium selenate had no effect. It is concluded that selenate and sulphate share a common pathway for transport across the human placental microvillus membrane.

INTRODUCTION

It is now widely accepted that selenium is an essential trace element. Rotruck et al. (1973) discovered that Se is a component of glutathione peroxidase (EC1.11.1.9), an enzyme which is believed to protect cells from the toxic effects of $H_2O_2$ and lipid hydroperoxides. It is beyond doubt that the human feto-placental unit requires an adequate supply of Se given that glutathione peroxidase has been identified in fetal tissues including the placenta (e.g. see Awasthi et al., 1977). Therefore an understanding of placental Se transport is important in relation to fetal nutrition.

There is now evidence which suggests that selenate ($SeO_4^{2-}$) shares a pathway for transport with sulphate across the brush border membrane of the human placenta. A study using isolated membrane vesicles has shown that $SeO_4^{2-}$ inhibits sulphate accumulation in a competitive manner (Shennan and Boyd, 1986a). Sulphate movement across this membrane has been found to be via an electroneutral DIDS-sensitive and Na-independent pathway (Boyd and Shennan, 1986a, b; Cole, 1984).

The purpose of this investigation was to measure directly the efflux of $SeO_4^{2-}$.
(using $^{75}\text{SeO}_4^{2-}$) from human placental microvillus membrane vesicles in order to establish whether selenate and sulphate efflux share any common properties.

**MATERIALS AND METHODS**

Normal term human placentae were placed on ice immediately after delivery of the baby and the process of preparing brush border membrane vesicles was normally started within 30 minutes. Microvillus membrane vesicles were prepared according to the method of Smith et al. (1974) as described by Boyd and Lund (1981). Membranes were suspended in a medium containing 1 mM Na$_2$SeO$_4$, 160 mM sucrose and 10 mM KOH-Hepes, pH 7.5. The vesicles were stored at $-70^\circ$C prior to use.

Selenate efflux was measured according to a method (used to measure sulphate efflux) previously described by Shennan and Boyd (1986b). Vesicles were loaded with $^{75}\text{SeO}_4^{2-}$ (supplied by Amersham International) by incubation for 90 minutes at 25°C ± 1°C in a medium containing 1 mM Na$_2$SeO$_4$, 160 mM sucrose and 10 mM KOH-Hepes pH 7.5. Following this loading period the extravesicular isotope was removed by passing the loading mixture through a column containing anion exchange resin (Dowex, Chloride-form, Mesh 50–100, 8 % cross linked). The vesicles were flushed through the column by washing with 2.5 mls of a solution containing 160 mM sucrose and 10 mM KOH-Hepes, pH 7.5. The eluent (which included the vesicles) was immediately split into aliquots and the efflux assay was initiated by adding 396/µl of vesicles to 924 µl of incubation media. At predetermined times 200 µl aliquots of reaction mixture were removed and applied to anion exchange columns. This mixture was eluted from the column by applying a further 2.5 mls of an appropriate ice-cold solution. The eluent was collected in vials and the radioactivity retained by the vesicles was counted using a Bertholf Mag 312 gamma counter.

Valinomycin at a concentration of approximately 20 nmoles/mg protein was used in all experiments. Since the intra- and extravesicular K concentrations were equal this effectively clamped the vesicle membrane potential to 0 mV.

The rate constant of selenate efflux, $K_t$, was calculated using the following equation:

$$K_t = \ln \left( \frac{S_t}{S_\infty} - 1 \right)$$

where $S_t =$ intravesicular selenate at time $t$ and $S_\infty =$ intravesicular selenate at infinite time (taken to be 120 minutes).

Like sulphate efflux, selenate efflux appears to follow first order kinetics following an initial rapid loss of selenate. This rapid loss may be due in part to a small fraction of ‘leaky’ vesicles.
RESULTS AND DISCUSSION

Figure 1 shows a typical time course of SeO\textsubscript{4}\textsuperscript{2-} efflux from human placental brush border membrane vesicles measured at 25°C and 4°C into a medium containing 1 mM Na\textsubscript{2}SeO\textsubscript{4}, 160 mM sucrose and 10 mM KOH-Hepes, pH 7.5. It is evident that lowering the temperature markedly decreases the rate of selenate efflux from the vesicles. In three experiments the rate constant of efflux was reduced from 0.0318 ± 0.0069 min\textsuperscript{-1} (± SE) at 25°C to 0.0066 ± 0.0007 min\textsuperscript{-1} (± SE) at 4°C. This amounts to a 79% reduction. Thus the temperature dependence of selenate efflux is similar to that

Fig. 1. The effect of temperature on SeO\textsubscript{4}\textsuperscript{2-} efflux from human placental microvillus membrane vesicles. Efflux was measured at 25°C (○) or 4°C (●) from vesicles suspended in a medium containing 1 mM Na\textsubscript{2}SeO\textsubscript{4}, 160 mM sucrose and 10 mM KOH-Hepes pH, 7.5.
Table I. A comparison of selenate and sulphate efflux rate constants

<table>
<thead>
<tr>
<th>Anion</th>
<th>Range</th>
<th>Mean ± SE</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeO₄²⁻</td>
<td>0.0209 - 0.0453</td>
<td>0.0357 ± 0.0023</td>
<td>11</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.0203 - 0.0547</td>
<td>0.0315 ± 0.0017</td>
<td>20</td>
</tr>
</tbody>
</table>

SeO₄²⁻ and SO₄²⁻ efflux were measured at 25 ± 1°C. For the SeO₄²⁻ efflux experiments the vesicles and incubation medium contained 1 mM Na₂SeO₄, 160 mM sucrose and 10 mM KOH-Hepes pH, 7.5. For the SO₄²⁻ efflux experiments vesicles and incubation medium contained 1 mM K₂SO₄, 160 mM sucrose and 10 mM KOH-Hepes pH, 7.5.

We have previously shown that the stilbene derivative DIDS is a potent inhibitor of sulphate efflux from human placental brush border membrane vesicles (Boyd and Shennan, 1986a). Therefore we decided to test the effect of this agent on selenate efflux. Table 2 shows the results of three experiments in which the effect of 2 x 10⁻⁴ M DIDS was studied. The addition of the drug to the incubation medium reduced the efflux rate constant by 83% from 0.0368 ± 0.0018 min⁻¹ (+SE) to 0.0062 ± 0.0009 min⁻¹ (+SE). Therefore it is apparent that DIDS inhibits SeO₄²⁻ efflux to the same extent as sulphate efflux.

Table 2. The effect of DIDS on selenate efflux

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No addition (control)</th>
<th>+ 2 x 10⁻⁴ M DIDS</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0382</td>
<td>0.0072</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>0.0332</td>
<td>0.0070</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>0.0389</td>
<td>0.0043</td>
<td>89</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.0368 ± 0.0018</td>
<td>0.0062 ± 0.0009</td>
<td>83 ± 3.1</td>
</tr>
</tbody>
</table>

SeO₄²⁻ efflux was measured from vesicles suspended in a medium containing 1 mM Na₂SeO₄, 160 mM sucrose and 10 mM KOH-Hepes pH, 7.5 with and without 2 x 10⁻⁴ M DIDS.

Chromate (CrO₄²⁻), which is an anion with a similar tetrahedral stereochemistry to both sulphate and selenate, has been shown to inhibit sulphate efflux from placental vesicles (Boyd and Shennan, 1986b). Therefore it was predicted that chromate should also inhibit selenate efflux. To test this prediction selenate efflux was measured into media with and without 10 mM CrO₄²⁻. The addition of this anion reduced the efflux rate constant by 66% from 0.0398 ± 0.0024 min⁻¹ (+SE) to 0.0132 ± 0.0018 min⁻¹.
Table 3. The effect of Chromate on selenate efflux

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No addition (control)</th>
<th>+10 mM CrO$_4^{2-}$</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0368</td>
<td>0.0123</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>0.0423</td>
<td>0.0109</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>0.0349</td>
<td>0.0184</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>0.0453</td>
<td>0.0110</td>
<td>76</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.0398 ± 0.0024</td>
<td>0.0132 ± 0.0018</td>
<td>66 ± 6.6</td>
</tr>
</tbody>
</table>

SeO$_4^{2-}$ efflux was measured from vesicles suspended in a medium containing 1 mM Na$_2$SeO$_4$, 190 mM sucrose and 10 mM KOH-Hepes pH, 7.5. When chromate was required the medium contained 1 mM Na$_2$SeO$_4$, 160 mM sucrose, 10 mM Na$_2$CrO$_4$ and 10 mM KOH-Hepes pH, 7.5.

Molybdate (MoO$_4^{2-}$), like chromate, inhibits sulphate efflux (but to a lesser extent) from placental membrane vesicles (Boyd and Shennan, 1986b). In a single experiment (results not shown) molybdate added to the incubation medium at a concentration of 10 mM inhibited selenate efflux by 23%. The inhibition of SeO$_4^{2-}$ efflux by CrO$_4^{2-}$ and MoO$_4^{2-}$ is probably a result of a slow rate of translocation of these two anions across the membrane following binding to the 'trans' aspect of the selenate transporter. Boyd and Shennan (1986b) failed to find any effect of medium sulphate on sulphate efflux from human placental brush border membrane vesicles. They concluded that the sulphate transporter does not serve as an obligatory sulphate self-exchanger. However there is indirect evidence that sulphate influx and efflux are mediated by a single transporter (Shennan and Boyd, 1986b). It is most likely that sulphate crosses this membrane in exchange for OH$^-$ ions or is co-transported with H$^+$ ions. On this basis it was predicted that SeO$_4^{2-}$ efflux should be independent of medium selenate. Selenate efflux was measured simultaneously into selenate-containing (10 mM) and selenate-free media. The rate constants of efflux into the respective media were 0.0408 ± 0.0064 (± SD, n = 2) and 0.0362 ± 0.0161 (± SD, n = 2). Therefore, as predicted, there was no appreciable difference in the selenate efflux rate constant measured into media containing or free of selenate.

The present results confirm that selenate and sulphate share a common transport pathway in the human placental brush border membrane. This conclusion is supported by the recent findings that selenate uptake by this membrane has similar kinetics to sulphate uptake, is inhibited competitively by sulphate and is DIDS-sensitive (Shennan, 1987).

Recently Wolffram et al. (1986) have shown using vesicles that there is a common transport mechanism for selenate and sulphate in the intestinal brush border membrane. However selenate uptake in their preparation, unlike placenta, is dependent on the presence of sodium ions in the incubation medium. This study and that of Wolffram et al. (1986) suggest that selenate metabolism may be greatly influenced by sulphate. This could be of importance if selenate is the optimal dietary selenium source and is the most abundant chemical form of selenium in plasma.
ACKNOWLEDGEMENTS

The author is grateful to Mrs Karen Pledge for her expert technical assistance.

REFERENCES